Bound gibberellins in *Prunus avium* L. cv. Mericier seeds.

Resumen. Se determinó la presencia de giberelinas libres y combinadas durante la estratificación en frío, en semillas de guindo dulce cultivar Mericier, sin endocarpo. Se probó el uso de las fosfomonoestearasas aplicadas después de someter los extractos a cromatografía en capa fina.

Ambas formas de giberelinas fueron encontradas en semillas en estado latente y estratificadas; en estas últimas la mayor actividad ocurrió después de 47 días de estratificación. Las giberelinas combinadas aumentaban su actividad en forma paralela a la de las giberelinas libres, sugiriendo que no hubo interconversión de la forma libre con la forma combinada. Por otra parte, semillas estratificadas en presencia del retardador del crecimiento AMO–1618 no mostraron una reducción significativa en su capacidad de germinación.

Mericier sweet cherry is a french cultivar introduced in Chile during the last century. Two types have been developed, black and red. The seed of the two types is characterized by the color of the endocarp. They differ in germination capacity and chilling requirement and both are extensively used as rootstocks (2). Little is known about the role of low temperature in breaking dormancy. One of the effects of stratification is the activation or release of several growth regulators such as gibberellins (3, 5, 6, 7). Proctor and Dennis (5), working on sweet cherry var. Schmidt, detected no changes in gibberellin-like substances in dormant versus non-dormant seeds. They also found no differences in germination capacity and in gibberellin content of the seeds.

The present study was undertaken to assess free and bound gibberellin content of sweet cherry seeds and to determine the possible connection of gibberellin content with the germination capacity of the seeds.

Materials and methods

Samples of seeds of red Mericier were obtained from a commercial orchard in Central Region of Chile, in 1978. The fruits were brought to the laboratory, and the seeds isolated and the endocarp removed. The isolated seeds were surface sterilized in 0.25% hypochlorite for 15 minutes, rinsed in sterile water and stratified in sterile sand or in filter paper (Whatman 3), moistened with distilled water or a solution of 1 mM or 0.1 mM or 0.1 mM of AMO–1618 for 90 days. Two samples of 30 or 50 seeds were removed after 0, 30, 47 and 90 days and analyzed for gibberellins. Also, two or three samples of 50 seeds were removed at each time to test germination capacity. For the gibberellin test, the seeds were immersed in methanol 80%, ground with a mortar and pestle and left overnight at 4°C. The supernatant was centrifuged at 2000 rpm and the residue evaporated under vacuum at 40°C. The residue was dissolved in 15 ml of phosphate buffer, pH 7.3, which was processed to give neutral hexane and ethyl acetate fractions, in conjuction with free and bound forms, according to the slightly modified method of Mielke and Dennis (1975).

Abbreviations: AMO–1618 4-hydroxy-5-isopropyl-2-methyphenyl trimethyl ammonium chloride piperedine carboxylate; GA_s gibberellins; GA_3 gibberellin A_3

Thin layer chromatography

Both free and bound fractions were spotted separately on pre-coated 20 x 20 cm glass plates coated with silicagel 60 F_{254} thickness 0.25 mm (E. Merck, Germany) and co-chromatographed with synthetic GA_3. The plates were developed with ethyl acetate-chloroform acetic acid (15:5:1). After developing the plate to 10 cm the chromatograms were divided into 5 equal zones, scraped and eluted with methanol. The eluates were evaporated to dryness and used for assay.

Bioassay

Phosphodiesterase or phosphomonoesterase activity was used as a bioassay to estimate the gibberellin content. To avoid slight variation in responses from one bioassay to the next, care was taken to test all Rfs of free and bound fractions of one experiment in the same run of an assay. Each Rfs to both free and bound fractions was dissolved in 1 ml of solution containing 250 ug streptomycin sulfate, 0.02 M $CaCl_2$ and embryoless barley seeds, shaken for 16-20 hours on a mechanical shaker in a stoppered test tube. Five milliliters of distilled water were added to each tube, which were throughly shaken and the supernatant centrifuged at 2000 rpm for 5 minutes. One milliliters of the extract was assayed for phosphomonoesterase activity. Typically 0.5 ml of 0.05 M sodium acetate buffer, pH 4.8 and 0.10 ml of bis-p-nitrophenyl phosphate (0.11 mg/ml) were incubated at 37°C for 10 minutes. After addition of 1 ml of NaOH 10% the bis-p-nitrophenyl produced was read at 405 nm in a spectrophotometer (Shimadzu UV 140–02 Kyoto, Japan).

Results and discussion

Seed germination was checked after 10 days of incubation at 20°C in the dark. AMO–1618 only slightly affected the germination capacity of the seeds after 90 days of chilling. However, 1 mM

Table 1. The effect of AMO–1618 on the germination capacity of sweet cherry endocarp-free seeds cultivar red Mericier, after 90 days of treatment at 5°C. Values are means of 3 samples of 50 seeds each.

Treatment	Germination (%)[1]
AMO– 1618	
10^{-4} M	80a
10^{-3} M	55b
Distilled water	64b
GA_3 10^{-4} M	100ac

1 Means followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

AMO–1618 slightly reduced germination from 64% to 54%. On the other hand, 0.1 mM AMO–1618 stimulated it (80% versus 65%) and GA_3 0.1 mM gave 100% (Table 1).

The distribution of gibberellin-like activity prepared from acidic ethyl acetate extract of whole sweet cherry seed on thin layer chromatography is shown in Figure 1. Both forms of GA_3 reached the highest level at 47 days of stratification and decreased to the level of dormant seeds after 90 days. Bound and free fractions paralleled their activity throughout the chilling period. Germination reached 94% by the end of the cold treatment.

The free gibberellin content of the whole seed, expressed in terms of gibberellin A_3 equivalent

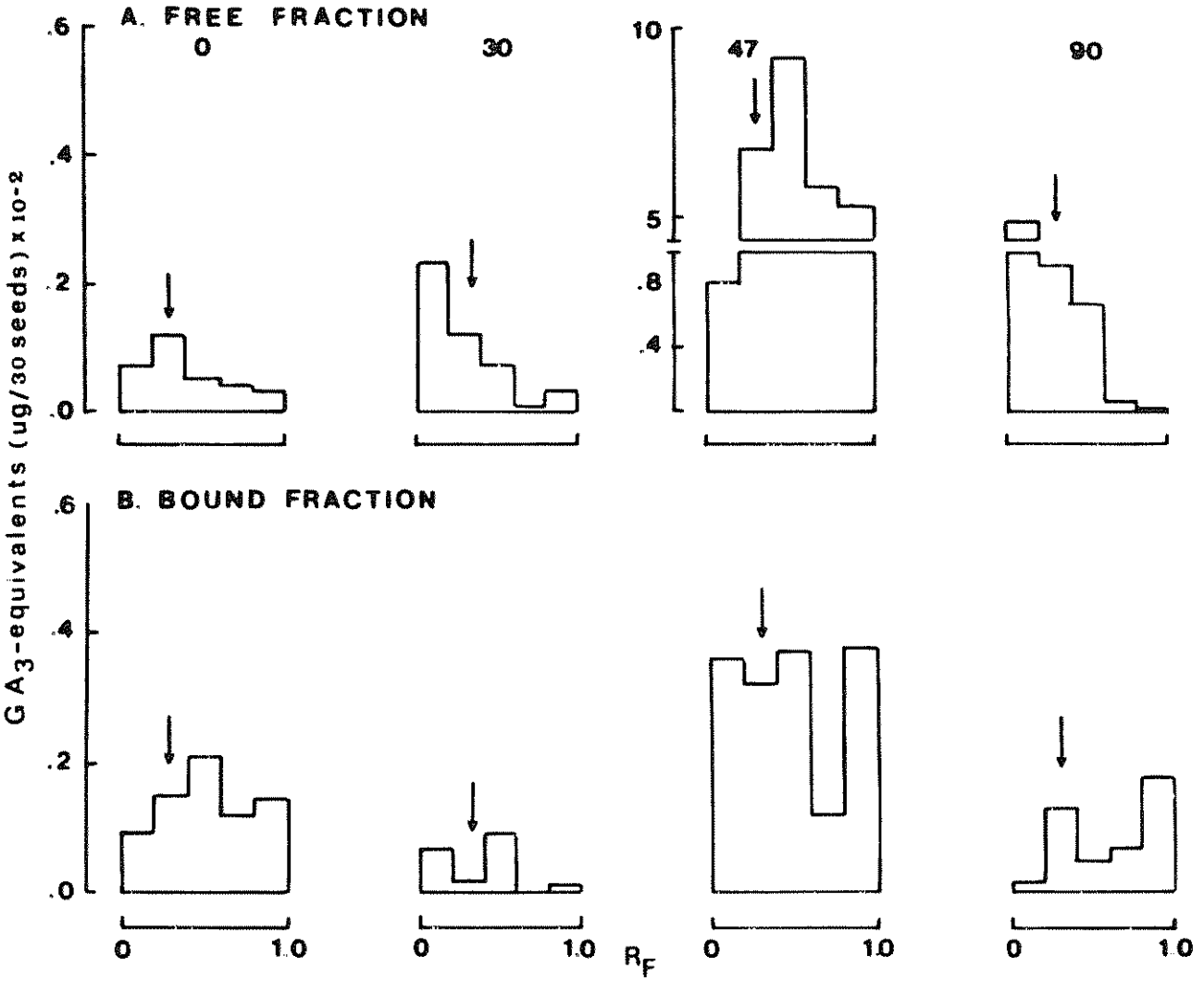

Fig. 1. Gibberellin-like activity in red Mericier sweet cherry at 0, 30, 47 and 90 days of stratification at 5°C. Detection was performed by measuring the activity of phosphodiesterase secreted by embryoless barley seeds after thin layer chromatography. A free form and B bound form. Arrows represented the Rfs of GA_3. Germination rates were 4% and 94% after 47 and 90 days respectively. Number on the top of each histogram represent time of stratification.

per 30 seeds at 0, 30, 47 and 90 days of chilling were 0.125 x 10^{-2}, 7.25 x 10^{-2} and 0.230 x 10^{-2} ug respectively. Thus, there was an approximately 60-fold increase of free gibberellin-like activity after 47 days, while a twofold increase in the bound form was observed in the material which showed very low (4%) germination.

The content of gibberellin-like substances in sweet cherry seeds undergoes changes during stratification, reaching a peak of activity after 47 days at 5°C for both free and bound forms. When seed germination capacity was very low (4%) the free form was predominant. This agrees with results reported for apple sees (6). However, AMO–1618 slightly reduced the germination ability of the seeds as has been observed for Golden Delicious apple seeds (1). The most characteristic and predominant of both fractions varied between Rf 0.2 – 1.0 reaching the highest level following 47 days of stratification. These results indicate no transformation of inactive bound gibberellin into free active form(s) during the chilling treatment. The same result has been reported for apple seeds (1).

The lack of effect of AMO–1618 in these seeds might be explained either assuming that the seeds have metabolized this substance or that the seeds are not sensitive to AMO–1618. This study did not confirm results reported by Proctor and Dennis (5). They reported no changes of GA_5 during stratification of sweet cherry seed var. Schmidt. This discrepancy may be due to either the use of a different variety or that the technique used to detect GA_5 activity which was not sensitive enough.

Experiments on the seed of *Prunus avium* Mericier indicated that gibberellin A_3 promotes the germination of sweet cherry when it was included in the stratification medium. However, as the dry storage period is prolonged not only gibberellins but also chilling fails to break the dormancy (unpublished results) of these seeds. Thus a stage is reached where the dormant seeds are unable to respond to both chilling and plant hormones.

Summary

Total free and bound forms of gibberellins were determined during stratification in endocarp-free seeds of sweet cherry cultivar Mericier using the phosphomonoesterase test applied after thin-layer chromatography.

Both forms of gibberellins were detected in dormant and after-ripened seeds and the highest levels of activity occurred after 47 days of stratification. Bound gibberellins parallel the activity of the free ones, suggesting no interconversion of the bound form into the free one. Seeds stratified in presence of the growth retardant AMO–1618 did not show significant reduction in the germination ability of the seeds.

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Faunistique et bioecologie des noctuelles (*Lepidoptera, Noctuidae*) des Antilles Françaises.

Summary. Noctuid moths constitute a serious pest problem in the French Antilles. Of the 127 species present, twenty are of agricultural concern. Noctuid moths are limiting corn production. The article discusses population dynamics, parasites and insect-plant relationships. A local corn ecotype seems more resistant than the imported variety, bred in Europe.

Les problèmes agronomiques posés par les Noctuelles aux Antilles Françaises s'avèrent extrêmement graves sur les cultures fourragères et vivrières. Ces insectes sont parfois de véritables facteurs limitant de l'introduction ou du développment de certaines cultures alternatives tel le mais. Cette plante, qui est extrêmement répandue en culture associée dans certaines îles de l'Archipel Antillais, peut s'avérer très intéressante dans le cadre de la diversification de l'agriculture de la Guadaloupe en particulier en association avec la canne à sucre.

Les données disponibles restent sur ce très vaste problème phytosantaire très fragmentaires et traitent d'observations éparses de pullulations des espèces les plus nuisibles à l'agriculture antillaise (1).

Nous présenterons plus particulièrement la faunistique de ces insectes, l'inventaire de leur biocénose parasitaire ainsi que la dynamique des populations des chenilles de deux espèces très nuisibles au mais: *Spodoptera frugiperda* A. et S. et *Heliothis zea* BODDIE.

Faunistique des Noctuelles des Antilles Françaises

Une liste, la plus complète, a été établie à partir des captures. Il a été tenu compte également des noctuelles de plusieurs collections de référence préexistantes. Ces observations intéressent toute la région Guadeloupe avec ses dépendances (Marie-Galante, la Désirade, les Saintes, St Martin et St Barthélémy) et la Martinique (Figura 1).

Les échantillonnages ont été effectués au piège lumineux ou par prélèvement des chenilles dans la nature pour élevage jusqu'à l'imago.

Au total, 127 espèces ont été recensées. Une partie est encore en cours d'identification ou de description pour les espèces nouvelles. Ces dernières seraient au nombre de trois (TODD, communication personnelle) dont une appartenant au genre *Eriotyga*

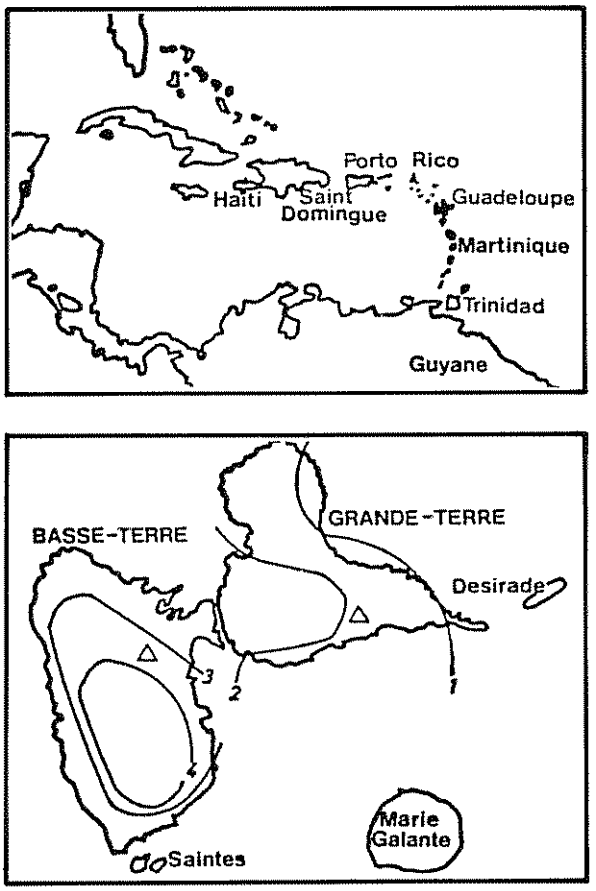

Fig. 1. Position de la Guadeloupe et de ses dépendances dans l'Archipel Antillais et zones ou ont été effectuées les plantations de maïs en Guadeloupe (Δ). Pluviométrie: 1 = inf. à 1 m; 2 = 1.50 m; 3 = 2.50 m; 4 = 3.50 m.